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(21) International Application Number: PCT/SE96/00891 (22) International Filing Date: 2 July 1996 (02.07.96) (30) Priority Data: 9502588-8 13 July 1995 (13.07.95) SE (71) Applicant (for all designated States except US): SCA MÖLNLYCKE AB [SE/SE]; S-405 03 Gothenburg (SE). (72) Inventors; and (75) Inventors/Applicants (for US only): RUNEMAN, Bo [SE/SE]; Jons väg 6, S-433 75 Jonsered (SE). ANDERSSON, Rolf [SE/SE]; Vallmovägen 11, S-435 31 Mölnlycke (SE). FORSGREN-BRUSK, Ulla [SE/SE]; Plommonvägen 35, S-435 43 Mölnlycke (SE). HOLM, Stig [SE/SE]; N Gimonäsvägen 25, S-907 38 Umeå (SE). GRAHN HÅKANSSON, Eva [SE/SE]; Nygatan 74, S-903 31 Umeå (SE). (74) Agents: AXELL, Kristina et al.; H. Albihs Patentbyrå AB, P.O. Box 3137, S-103 62 Stockholm (SE).		(81) Designated States: AU, CA, CN, CZ, HU, IL, JP, MX, NO, NZ, PL, SK, TR, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE). Published <i>With international search report.</i>
(54) Title: INHIBITING THE GROWTH OF BACTERIA IN ABSORBENT ARTICLES BY ADDING OTHER BACTERIA (57) Abstract The invention relates to absorbent articles, such as diapers and like articles and is concerned with methods for preventing undesirable odours and/or preventing the growth of undesirable microorganisms when the articles are in use, and also provides an absorbent article which can be worn for long periods of time without generating undesirable odours, incurring the risk of infection or having a negative effect on skin. Another object is to amplify the presence in the wearer's urogenital zone of microbiological flora that will assist in preventing the occurrence of urinal tract infections. These objects have been achieved by adding to the absorbent articles microorganisms which exhibit antagonistic properties against present undesirable strains of microorganisms, so as to restrain the growth of these or establishing of new undesirable species.		

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**INHIBITING THE GROWTH OF BACTERIA IN
ABSORBENT ARTICLES BY ADDING OTHER BACTERIA**

TECHNICAL FIELD

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The present invention relates to absorbent articles such as
diapers, incontinence guards, sanitary napkins and like articles,
and is concerned with methods of preventing the generation of
undesirable odours and/or the growth of undesirable microorganisms
10 as the article is worn.

PROBLEMS

Many designs of absorbent articles of this kind are known to the
15 art. Conventionally, the absorbent body of such articles is
produced by dry-defibering cellulose pulp contained for instance in
rolls, bales or sheets, and transforming the fluffed pulp to a pulp
mat, sometimes while admixing so-called superabsorbents, which may
be polymers capable of absorbing several times their own weight in
20 water or body fluid.

The absorbent body may also include other components, for instance
components that will improve the ability of the absorbent body to
take-up and disperse liquid, and that will increase its coherency
25 and its ability to resist deformation in use.

Articles of this kind are liable to generate undesirable odours
when in use, caused among other things by microbial metabolism,
biological or chemical decomposition of components in body fluids,
30 such as urine or menstruation fluid, for instance.

Another problematic area associated with the use of absorbent
articles is the risk of infection caused by pathogenic microorga-
nisms.

35

Another problem is found in the working environmental risks that
can arise when handling soiled diapers that contain a large
quantity of microorganisms. The growth of microorganisms that can

take place in a soiled, used diaper during its storage after use can contribute to elevated odour problems and can also increase the risk of spreading undesirable microorganisms.

- 5 Another problem is the different forms of skin irritation and skin infections that can be caused directly or indirectly by microorganisms.

Microorganisms or their products that are known to contribute to
10 the occurrence of undesirable odours, to cause infections in the urinal tract or to be associated with the occurrence of skin problems are, for instance such microorganisms as Proteus, Pseudomonas, Escherichia, Klebsiella, Enterococcus, Staphylococcus, Streptococcus and Candida.

15

DESCRIPTION OF THE KNOWN PRIOR ART

Different methods of alleviating the aforescribed problems have been proposed. The International Patent Applications WO 91/11977
20 and WO 91/12031 describe methods of adsorbing undesirable odours in zeolite included in the absorbent article. U.S. Patent 4,385,632 describes the addition of copper salt to a diaper with the intention of preventing the decomposition of urea to ammonia and therewith prevent the occurrence of unpleasant smells.

25

U.S. Patent 3,794,034 describes the significance of pH in an absorbent article, and the significance of impregnating the article with a buffering substance by means of which the pH of the article can be maintained at between 3.5 and 6.0, which is advantageous
30 from both the aspect of inhibiting the growth of undesirable bacteria, and therewith the generation of undesirable odours, and in avoiding a negative effect on the skin. European Patents EP 202127 and EP 311344 describe the adjustment of pH in absorbent articles that include superabsorbent material. Since buffering
35 substances can have a negative effect on many superabsorbent materials, it is necessary to take separate measures to avoid undesirable effects. In the case of European Patent EP 202127, the superabsorbent material and buffering substances are placed in

mutually separated zones in the absorbent article. In the case of European Patent EP 311344, the buffering and the superabsorbent properties have been combined in one and the same material, and a separate bacteria-inhibiting substance has also been added.

5

The drawback with these described methods is that when only an odour-absorbing agent is added, the bacteria are still able to grow, and the bacteria inhibiting agents, which are often selective, can create risks, for instance, in the form of allergenic properties or negative ecological consequences when handling waste. Furthermore, the use of this type of agent involves the risk that resistant strains will occur.

It is known within the medicine and foodstuff technologies to use bioconservation with the aid of bacterial antagonism as a conserving method, and to inoculate special bacteria strains to favourize bacteria populations that are beneficial to the stomach and intestines, for health-promoting purposes. Examples in this respect are conventional yoghurt and soured milk, and also novel bioactive foodstuffs. This methodology also includes the use of bacteria such as so-called probiotic bacteria as a substitute for antibiotic bacteria.

Canadian Patent CA 1,298,556 teaches the medical use of selected strains of lactobacteria, wherein, among other things, whole cells or cell fragments of Lactobacillus are used to treat or preclude the occurrence of urinal tract infections. International Patent Application WO 93/09793 describes the use of lactobacteria and skimmed milk preparations for precluding or preventing urogenital infections. International Patent Application WO 92/13577 describes a tampon or sanitary napkin that has been impregnated with a culture of lactic-acid producing bacteria, preferably of the genus *Pediococcus*, isolated from healthy individuals. The tampon or sanitary napkin is intended for the prophylactic treatment of urogenital infections.

SUMMARY OF THE INVENTION

The object of the present invention is to provide an absorbent article of the kind mentioned in the introduction which will allow
5 the article to be worn, even for a long period, without permitting microorganisms to grow or to become active to an extent such as to promote undesirable odours, to incur the risk of infection or to have a negative effect on the skin. Another object of the invention is to enable antagonistic microorganisms to be transferred to the
10 wearer so as to amplify in the wearer's urogenital zone the occurrence of such microbiological flora as those that will assist in preventing the occurrence of urinary tract infection. These objects have been achieved in accordance with the invention by adding to the absorbent article microorganisms which exhibit
15 antagonistic properties against present undesirable strains or arising undesirable strains of microorganisms present in the absorbent article or in the urogenital zone of the wearer during regular use of the absorbent article, wherein said microorganisms are added in an amount and have an activity such as to restrain the
20 growth of undesirable species or establishing of new undesirable species of microorganisms.

BRIEF DESCRIPTION OF THE DRAWING

25 Fig. 1 illustrates in terms of percentage the proportion of different bacteria strains that exhibit inhibited growth in the presence of antagonistic strains.

DETAILED DESCRIPTION OF THE INVENTION

30

The object of the present invention is to provide an absorbent article of the kind mentioned in the introduction which will enable the article to be worn for a relatively long period of time without microorganisms being allowed to grow to an extent in which
35 undesirable odours are generated, in which the risk of infection is created, or to an extent which will have a negative effect on skin. A further object of the invention is to enable antagonistic microorganisms to be transferred to the wearer so as to amplify in

the wearer's urogenital zone the presence of microbiological flora that will assist in preventing the occurrence of urinal tract infections.

- 5 Bacteria that cause unpleasant smells may belong to, for example, the family Enterobacteriaceae, e.g. *Proteus mirabilis*, *Proteus vulgaris*, *Escherichia coli* and *Klebsiella*.

Bacteria that cause urinal tract infections may belong to, for
10 example, the families Enterobacteriaceae and Micrococcaceae or the genus *Streptococcus*. Examples of species and genera are *Escherichia coli*, *Proteus mirabilis*, *Enterococcus*, *Klebsiella*, *Staphylococcus* and *Streptococcus*.

- 15 Microorganisms associated with skin infections are Ascomycetes, Pseudomonadaceae and Micrococcaceae and the genus *Streptococcus*, e.g. *Candida albicans*, *Pseudomonas*, *Staphylococcus* and *Streptococcus*.

- 20 The invention is based on microbiological antagonism. This implies that one microorganism or combinations of microorganisms inhibits/inhibit other microorganisms by competing for substrate, changing pH, forming enzymes, toxins, carbon dioxide, peroxides or antibiotics, so-called bacteriocines.

25

Antagonistic microorganisms may be naturally occurring microorganisms which are non-toxic and do not have any negative biological effect on humans, in the form of infections or skin changes.

- 30 Antagonistic microorganisms may also be produced biotechnically.

The addition to an absorbent article of microorganisms which exhibit antagonistic properties against such undesirable strains of microorganisms that are present when the absorbent article is worn
35 regularly, is able to restrain the growth of these undesirable strains of microorganisms or establishing of new undesirable strains. Even some killing of microorganisms of undesirable species may occur. It is necessary for the microorganisms to have an

activity and to be added in amounts which will achieve the desired effect. Normally, this effect is achieved when the number of antagonistic microorganisms per absorbent article exceeds 10^6 cfu, preferably 10^8 cfu, more preferably 10^9 cfu. By regular use is meant
5 in this case daily use of an article with the article replaced several times during a calendar day, as is the case with products intended for use with children or incontinent adults. The term regular use may also include the use relevant to sanitary napkins or tampons during a menstruation period.

10

One advantage afforded by the use of antagonistic microorganisms is that there is avoided an undesired selection pressure on the micro environment, such as favouring potential disease-promoting microorganisms and therewith the risk of developing pathogenic
15 strains that are resistant to antibiotics and chemopharmaceutical preparations. Since the antimicrobial system is based on a natural, biological process, there is less risk of environmental ecological and toxic disturbances.

20 An antagonistic strain shall exhibit a growth-inhibiting effect on several of the aforesaid undesired microorganisms, with conventional interference techniques.

A desired antagonistic microorganism shall also be capable of
25 surviving in storage and to retain its growth ability or its ability to retain its activity in the absorbent article when worn.

The microorganisms that exhibit antagonistic properties may be bacteria or other microorganisms, for instance fungi. When the
30 antagonistic microorganisms are bacteria, these bacteria are preferably selected from the family Lactobacillaceae and particularly from the genera Lactobacillus or Lactococcus and preferably from the species Lactobacillus acidophilus, Lactobacillus curvatus, Lactobacillus plantarum or Lactococcus
35 lactis.

An absorbent article produced in accordance with the invention may include a permeable outer sheet which is intended to lie proximal

to the wearer in use, a preferably liquid-impermeable backing sheet which is intended to lie distal from the wearer in use, and an absorbent structure placed between the outer sheet and the backing sheet. In some cases, an additional sheet in the form of, e.g.,
5 wadding or like material, may be placed between the outer sheet and the absorbent structure. The microorganisms exhibiting antagonistic properties may be placed in different parts of the absorbent article, for instance in the outer sheet, in the absorbent structure of the absorbent article, between two of the layers in
10 the absorbent article, in a loose insert product in the absorbent article, or in some other way.

The following Examples illustrate the effect of antagonistic bacteria strains in more detail.

15

Example 1

Synthetic urine to which a microorganism growth medium had been added was used as a test liquid. The synthetic urine contained
20 monoions, divalent ions, cations and anions and urea and was prepared in accordance with information contained in Geigy, Scientific Tables, Vol. 1, 8th ed., 1981, p. 53. The microorganism growth medium was based on information concerning Hook and FSA media for enterobacteria.

25

Example 2

Tests were carried out in accordance with the "agar overlay" method with the intention of studying bacterial antagonism. The method is
30 based on the growth-inhibiting substance produced by the lactobacteria diffusing through an agar layer and inhibiting the growth of the test organisms.

Lactic acid bacteria, five strains of Lactobacillus and three
35 strains of Lactococcus, were cultivated to an overnight culture in a suitable broth. Lactococcus were cultivated in M17 and Lactobacillus were cultivated in MRS. Agar (2%) of M17 and MRS (25 ml) respectively were mixed with 1.0 ml of respective bacteria and

moulded in a petri dish. The Agar plates were incubated overnight at 37°C. The plates that contained MRS were incubated in a CO₂ atmosphere. Reference plates were prepared in a corresponding manner, but without lactic acid bacteria. A further layer containing 25 ml agar was moulded on top of the layer present in the petri dishes and allowed to solidify.

The test organisms, in the form of Gram-negative bacteria of respectively *Escherichia coli*, *Klebsiella* spp and *Proteus* spp, and 100, 91 and 50 strains respectively, were cultivated in a broth and a dilution corresponding to 10⁷ cfu/ml was prepared in a Bertani tray. The test bacteria were then stamped on the new agar layer with the aid of a so-called steers steel pin replicator. The plates were incubated at 37°C for twenty-four hours. At the end of the incubation period, the plates were scanned and compared with the reference plates. "Growth", "inhibition" and "zero growth" were registered for respective test organisms when scanning the plates. All agar layers were measured with regard to pH, and plates having a pH beneath 5.0 were retested with pH-adjusted agar.

20

The results are listed in Tables 1-6 and shown in Fig. 1. The total number of test organisms that had been inhibited or given zero growth were calculated in percentage units. The results show that the growth of respective test organisms *Escherichia coli*, *Klebsiella* and *Proteus* was greatly inhibited by the presence of genera *Lactobacillus* while some inhibition was obtained in the presence of genera *Lactobacillus*.

Test organism *Escherichia coli*

Lactococcus	No.	Growth number	Inhibition number	Zero growth number	Inhibition or zero growth
					%
30	L 3	85	14	1	15
	L 4	91	9	1	10
	L 5	65	7	28	35
	L 9	4	0	96	96
	L 26	0	0	100	100

Table 1

Test organism Escherichia coli

	Lactobacillus	Growth	Inhibition	Zero growth	Inhibition or
	No.	number	number	number	zero growth
5					%
	LB 13	0	0	100	100
	LB 14	0	2	98	100
	LB 16	0	2	98	100

Table 2

10

Test organism Klebsiella spp

	Lactococcus	Growth	Inhibition	Zero growth	Inhibition or
	No.	number	number	number	zero growth
					%
15	L 3	68	16	7	25
	L 4	71	23	6	32
	L 5	64	10	17	29
	L 9	51	10	30	44
	L 26	34	36	21	63

20

Table 3**Test organism Klebsiella spp**

	Lactobacillus	Growth	Inhibition	Zero growth	Inhibition or
	No.	number	number	number	zero growth
25					%
	LB 13	0	0	91	100
	LB 14	62	22	7	32
	LB 16	0	0	91	100

Table 4

Test organism Proteus spp

	Lactococcus	Growth	Inhibition	Zero growth	Inhibition or
	No.	number	number	number	zero growth
					%
5	L 3	47	2	1	6
	L 4	49	1	0	2
	L 5	39	7	4	22
	L 9	45	4	1	10
	L 26	36	8	6	28

10

Table 5**Test organism Proteus spp**

	Lactobacillus	Growth	Inhibition	Zero growth	Inhibition or
	No.	number	number	number	zero growth
					%
15	LB 13	0	0	50	100
	LB 14	10	19	21	80
	LB 16	2	7	41	96

Table 6

20

Example 3

Five test products were produced, each consisting of a pulp body that comprised a fibre mixture of 50% chemithermomechanical cellulose pulp and 50% chemical cellulose pulp with an addition of about 5% superabsorbent material. The pulp body was sandwiched between a permeable nonwoven material having a surface weight of 23 g/m² and an impervious backing sheet comprised of 33 µm polyethylene film. A layer of polyester wadding having a surface weight of 65 g/m² was placed between the nonwoven sheet and the pulp body. A mixture of freeze-dried lactobacteria of genus Lactobacillus was placed between the wadding and the pulp body in an amount corresponding to 10⁸ bacteria per test product. 50 ml of synthetic urine according to Example 1 were then applied to the pulp body. The presence of lactobacteria in the groins and at the mouth of the urethra of five test subjects was measured, whereafter the subjects wore respective test products for two hours in the manner of a diaper or sanitary napkin. The presence of lactobac-

teria in the groins and at the mouth of the urethras of the test subjects was then again measured. The results are set forth in Table 7. It will be seen from the Table that the freeze-dried lactobacteria were activated when the test products were worn by the test subjects and that these lactobacteria were also transferred to the test subjects.

		Start value	Start value	After 2 hrs.	After 2 hrs.
		urethra	groin	urethra	groin
10		number	number	number	number
	Test	lacto-	lacto-	lacto-	lacto-
	person	bacteria	bacteria	bacteria	bacteria
	No. 1	No growth	No growth	3.6×10^3	1.5×10^4
15	No. 2	No growth	No growth	2.8×10^3	2.7×10^2
	No. 3	No growth	No growth	1.4×10^3	8.4×10^2
	No. 4	No growth	No growth	3.8×10^6	4.0×10^3
	No. 5	No growth	No growth	4.5×10^3	8.6×10^2

Table 7

20

It will be understood that the invention is not restricted to the illustrated and described exemplifying embodiment thereof and that other embodiments are conceivable within the scope of the following Claims.

CLAIMS

1. An absorbent article, such as a diaper, a sanitary napkin, a tampon or the like to which microorganisms have been added,
5 characterized in that the addition of microorganisms is selected so that when the absorbent article is worn regularly for a short or longer period, the microorganisms will exhibit antagonistic properties against present undesirable strains or arising undesirable strains of microorganisms present in the absorbent
10 article or in the urogenital zone of the person wearing said article; and in that the microorganisms are added in such quantities and have such activity as to restrain the growth of undesirable species or establishing of new undesirable species of microorganisms.
- 15 2. An absorbent article according to Claim 1, characterized in that the microorganisms that exhibit antagonistic properties are bacteria.
- 20 3. An absorbent article according to Claim 2, characterized in that the antagonistic bacteria are taken from genera *Lactobacillus* or *Lactococcus* having antagonistic properties.
- 25 4. An absorbent article according to Claim 3, characterized in that the antagonistic bacteria are taken from species *Lactobacillus acidophilus*, *Lactobacillus curvatus*, *Lactobacillus plantarum* or *Lactococcus lactis*.
- 30 5. An absorbent article according to Claim 1, characterized in that the number of antagonistic microorganisms per absorbent article exceeds 10^6 cfu, preferably 10^8 and more preferably 10^9 cfu.
- 35 6. An absorbent article according to claim 1, characterized in that the microorganisms are such that they exhibit antagonistic properties against one or several undesirable strains from the families Enterobacteriaceae, Micrococcaceae, Pseudomonadaceae and Ascomycetes and the genus Streptococcus, preferably from the species *Proteus mirabilis*, *Proteus vulgaris*, *Escherichia coli*,

Candida albicans and the genera Klebsiella, Enterococcus, Staphylococcus, Streptococcus and Pseudomonas.

7. An absorbent article, such as a diaper, sanitary napkin, 5 tampon or like article which includes a permeable outer material which is intended to lie proximal to the wearer in use, a preferably liquid-impermeable backing material which is intended to lie distal from the wearer in use, and an absorbent structure placed between the surface material and the backing material, wherein the 10 absorbent article also includes microorganisms, characterized in that the microorganisms have been selected so that when the absorbent article is used regularly for a short or longer period, the microorganisms will exhibit antagonistic properties against present or arising undesirable strains of microorganisms present in 15 the absorbent article or in the urogenital zone of the person wearing said article; and in that said microorganisms are added in such quantities and have such activity as to restrain the growth of undesirable species or establishing of new undesirable species of microorganisms.

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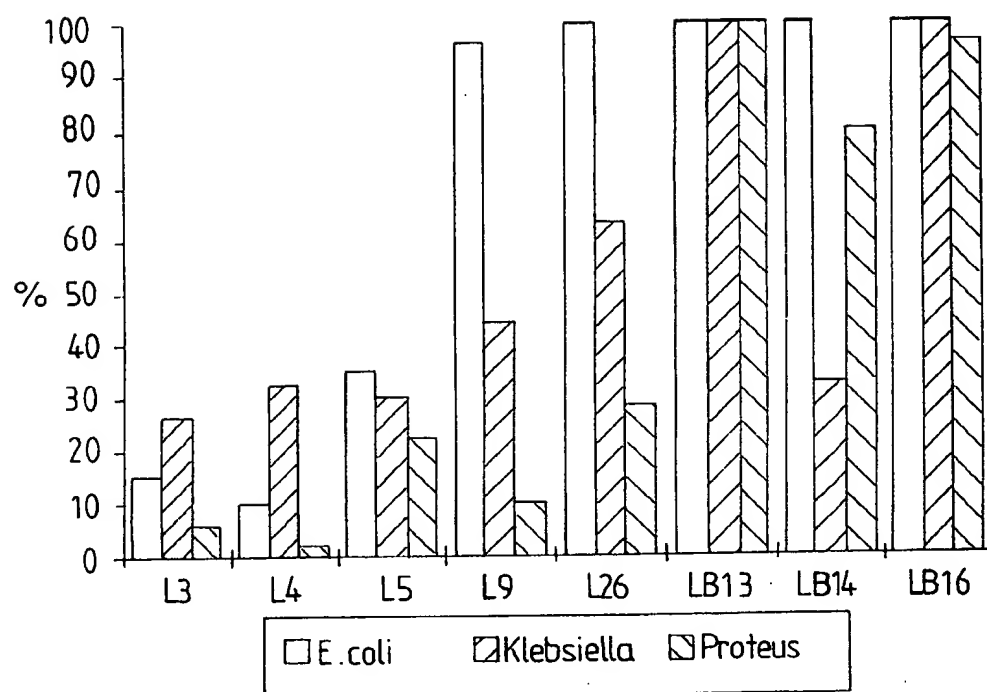
8. An absorbent article according to Claim 7, characterized in that the microorganisms exhibiting antagonistic properties are placed in the surface material of the absorbent article.

25 9. An absorbent article according to Claim 7, characterized in that the microorganisms exhibiting antagonistic properties are placed in the absorbent structure of the absorbent article.

10. An absorbent article according to Claim 7, characterized in 30 that the microorganisms exhibiting antagonistic properties are placed in a loose insert product in the absorbent article.

11. An absorbent article according to Claim 7, characterized in that the microorganisms exhibiting antagonistic properties are 35 placed between two of the layers in the absorbent article.

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/SE 96/00891

A. CLASSIFICATION OF SUBJECT MATTER		
IPC6: A61L 15/36, A61F 13/15 According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED		
Minimum documentation searched (classification system followed by classification symbols)		
IPC6: A61L, A61F		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
SE,DK,FI,NO classes as above		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SE 8505491-4 A (HANDELSBOLAGET PER OCH PIA EDLUND), 21 May 1987 (21.05.87), claims 1 and 4 ---	1-11
X	DE 2309575 A1 (WENDERLEIN, J. MATTHIAS), 5 Sept 1974 (05.09.74), claims 1,2 and 4 -----	1-11
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
SE-A- 8505491-4	21/05/87	NONE	
DE-A1- 2309575	05/09/74	NONE	